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THE ANIMAL ORGANISM

A STUDY OF SOME POSSIBLE PRECURSORS OF LYSINE

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DANIEL A. MCGINTY

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIRE-
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THE JOURNAL OF BIOLOGICAL CHEMISTRY,
Vol. LXII, No. 1, PAGES 75-92, NOVEMBER, 1924)

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AMINO ACID SYNTHESIS IN THE ANIMAL ORGANISM. THE AVAILABILITY OF SOME CAPROIC ACID DERIVATIVES FOR THE SYNTHESIS OF LYSINE.*

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(Received for publication, August 1, 1924.)

In the functioning of body cells, amino acids, which are carried to the cells by the blood stream and lymph after absorption from the alimentary canal or which are stored in the cell protoplasm as protein, are constantly catabolized. This process varies in its intensity; the amino acid may be completely broken down to water, carbon dioxide, and ammonia, apparently furnishing energy only, or a partial degradation may result in a conversion of fragments of the amino acids into products having a vital function in the various processes of the body. Thus, the tyrosine liberated in the cells presumably gives rise to adrenalin; tryptophane is believed to be necessary for the formation of thyroxin, the active principle of the thyroid; and cystine is probably essential for the synthesis of glutathione which is of such vital importance in oxidative processes in the body. If these amino acids are not furnished to the cell they must be synthesized from other metabolites present and if this is not possible, malnutrition results, growth ceases, reproduction is interfered with, and death may follow.

If the growing animal be furnished, not with the amino acid itself, but with certain closely related derivatives of that amino acid, the other nutritional requirements being satisfactory, can it synthesize the necessary unit from the derivatives or can it

* An abstract of a thesis submitted by Daniel A. McGinty in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Michigan.

utilize the derivatives themselves for growth and development? Such a study is presented in the present attempt to determine whether the organism of the young white rat may utilize certain amino and hydroxy derivatives of caproic acid closely related to lysine (α , ϵ -diaminocaproic acid) as precursors of lysine for growth.

The validity of the experimental method used in these studies is conditioned by the indispensability of lysine for normal growth of the young white rat. The most decisive evidence of the rôle of lysine in nutrition is that of Osborne and Mendel (1, 2) in which they demonstrate that gliadin, the alcohol-soluble protein of wheat gluten, as the only source of protein in the diet of young rats, did not permit growth. The animals, however, maintained their body weight at quite constant levels. The failure of growth was presumably due to the low lysine content (1.21 per cent) of gliadin (3), although this lysine content was apparently sufficient for maintenance. Gliadin, when supplemented with lysine, allowed practically normal growth, thus proving that gain in weight is dependent on the presence of lysine, and that lysine is a limiting factor in the amino acid content of gliadin. Experiments with zein (1), lacking in tryptophane and lysine, as the source of protein, have also demonstrated the necessity of lysine for growth. Hart, Nelson, and Pitz (4) have proven that the mammary gland of the lactating female rat is unable to synthesize lysine. Buckner, Nollau, and Kastle (5) fed chicks on grains high in lysine. Those on the lysine-deficient grains showed decidedly slower growth and development. Osborne and Mendel (6) obtained results agreeing with those of Buckner, Nollau, and Kastle.

Abderhalden (7, 8) demonstrated that the use of hydrolyzed casein, from which lysine had been removed, as a source of protein for adult dogs led to a negative nitrogen balance which was slightly improved if lysine was returned to the diet. Abderhalden (9) also found that body weight of an adult rat fell rapidly if lysine was lacking from the synthetic diets, but that this loss in weight was regained when lysine was again incorporated into the food. Other investigators confirm the views of the indispensability of lysine, although Geiling (10) does not agree with the opinion of Hart, Nelson, and Pitz (4) that lysine is necessary

for adult maintenance. McCollum, Simmonds, and Pitz (11) agree that lysine is necessary for growth, although they do not state that it is indispensable for adult maintenance.

The possibility of lysine synthesis from simpler caproic acid derivatives has not been extensively studied. Embden and Schmitz (12) have demonstrated by perfusion experiments with the surviving liver that α -hydroxy and α -keto acids may be converted to the corresponding amino acids. That this reaction which can readily be demonstrated in perfusion experiments also occurs in the intact living organism has not been proven, although some evidence in support of this may possibly be found in the experiments of Grafe and Schl pfer (13) and Abderhalden (14).

Of the various hydroxy and amino derivatives of caproic acid of interest in connection with the problem of lysine synthesis, the following were available for the present study, α -hydroxy-, ϵ -hydroxy-, ϵ -amino-, and α -hydroxy- ϵ -aminocaproic acids. In addition, nor-leucine (α -aminocaproic acid) has already been shown by one of us (15) to be unavailable as a lysine precursor. Of these derivatives the synthesis of lysine from α -hydroxy- ϵ -aminocaproic acid seemed most probable in the light of other studies. The only chemical change necessary to form lysine from α -hydroxy- ϵ -aminocaproic acid would be a reversal of the hydrolytic deamination process which is thought by some to be possible in the living organism (12, 13). Likewise, α -amino- ϵ -hydroxycaproic acid might be considered to be easily converted into lysine by a reversal of the deamination process on the ϵ carbon atom. If both the α and ϵ carbon atoms are capable of amination by union of the hydroxy groups with ammonia, then α , ϵ -dihydroxycaproic acid should likewise be capable of lysine synthesis. Considering those derivatives in which amination would have to take place by replacement of a hydrogen atom, possibility of synthesis is less likely. This is especially true in view of the work of Lewis and Root (15) who attempted to replace lysine by nor-leucine in the food of growing rats. They fed diets in which gliadin formed the protein fraction. In the case of nor-leucine, the α -amino group is already present in the molecule, and since there is some evidence that the ϵ -amino group of lysine does not function in the peptide linkage, it was

thought that the animal organism might utilize nor-leucine without further substitution of amino groups in the formation of new protein available for growth. They found, however, that neither the inactive *dl* form nor the active *d*-nor-leucine was able to supplement gliadin as lysine does, indicating that there was neither a synthesis of lysine from nor-leucine, nor a utilization of nor-leucine in place of lysine. Since nor-leucine is not to be considered as a precursor of lysine, one must probably exclude ϵ -aminocaproic acid, α -hydroxycaproic acid, and ϵ -hydroxycaproic acid on the same grounds, that amination of a carbon atom, without the hydroxyl group as an intermediate stage, is apparently not possible.

EXPERIMENTAL.

With one exception (Rat 69, Chart IV), the experimental animals used were young white rats which were placed on the experimental diets at an age of about 5 weeks when the weight was from 45 to 65 gm. In general, the laboratory procedure described by Miss Ferry (16) of the Osborne and Mendel laboratory was employed. The rats were bred in the laboratory. No disease occurred in the colony during the course of the experiments. The casein used as a food for control animals was a commercial product (Lister Bros.' white casein), which was extracted twice with hot alcohol for 30 minutes to remove lipoids partially. Gliadin was prepared in the laboratory. The inorganic salt mixture used was that of Osborne and Mendel (17). The corn-starch and lard were commercial products, the same brands being used throughout. The water-soluble vitamin was furnished by a yeast vitamin powder (Harris Laboratories), approximately 50 mg. of this powder being fed daily in the form of a pill made with equal parts of vitamin powder and starch. This vitamin preparation contained no protein and supplied little nitrogen. An analysis of this powder made by Miss M. L. Long of this laboratory showed the following composition: nitrogen, 8.11 per cent; sulfur, 0.71 per cent; phosphorus, 5.43 per cent; ash, 24.11 per cent; moisture, 7.04 per cent; amino nitrogen (Folin) before hydrolysis, 1.66 per cent, after hydrolysis, 2.37 per cent; cystine (Folin and Looney) before hydrolysis, 1.18 per cent, after hydrolysis, 1.46 per cent. The fat-soluble vitamin was supplied by cod liver oil.

Lysine was prepared in the laboratory by acid hydrolysis of casein by the method of Kossel and Kutscher described by Weiss (18). This method was modified in that the first neutralization was made with calcium hydroxide instead of barium hydroxide. The lysine picrate of Kossel and Kutscher was converted into the hydrochloride by extraction of a dilute hydrochloric acid solution of the picrate with ether to remove the picric acid. The extracted liquid was then evaporated on a water bath to dryness, dissolved in methyl alcohol, and precipitated as lysine dichloride

TABLE I.

Composition of Diets and Values of Caproic Acid Derivatives in Terms of Gliadin.

	18 per cent protein.	15 per cent protein.	12 per cent protein.	9 per cent protein.
	gm.	gm.	gm.	gm.
Protein.....	18.0	15.0	12.0	9.0
Salt mixture.....	4.5	4.5	4.5	4.5
Sucrose.....		3.0	4.0	4.5
Starch.....	50.0	50.0	52.0	54.5
Lard.....	24.5	24.5	24.5	24.5
Cod liver oil.....	3.0	3.0	3.0	3.0
Total.....	100.0	100.0	100.0	100.0

Equivalent nitrogen values of the caproic acid derivatives added to gliadin diets.

1 gm. of lysine (1.486 gm. of lysine dichloride) replaced 1.2 gm. of gliadin.

1 " " ϵ -aminocaproic acid replaced 0.66 gm. of gliadin.

1 " " α -hydroxy- ϵ -aminocaproic acid replaced 0.60 gm. of gliadin.

1 " " cystine replaced 0.66 gm. of gliadin.

by absolute ethyl alcohol. Lysine was fed as the dihydrochloride. In certain experiments, synthetic *dl*-lysine was used to supplement gliadin. The caproic acid derivatives studied and the synthetic *dl*-lysine were prepared under the direction of one of us (M.) in the Laboratory of Organic Chemistry of the University of Illinois. An account of their preparation and properties will be published elsewhere.¹

The food mixtures were made up frequently so as to insure freshness and kept in an ice box. In those experiments in which lysine or caproic acid derivatives were incorporated in the diet along with the gliadin, a portion of the gliadin, equivalent in

¹Marvel, C. S., *J. Am. Chem. Soc.*, 1924, xl (in press).

its amount of nitrogen to the nitrogen of the lysine or caproic acid derivatives added, was removed. In this way the total nitrogen content of the diet remained the same. In Table I is presented the composition of the various diets with the equivalents in terms of gliadin of lysine and the caproic acid derivatives fed.

Rats were always given gliadin diets previous to addition of caproic acid derivatives in order to demonstrate in each rat the failure of growth on such a diet. Control experiments with casein were made on each litter of rats to demonstrate the possibility of normal growth with this adequate protein. In all cases where caproic acid derivatives were used, lysine subsequently replaced the derivative in question to demonstrate the adequacy of a gliadin plus lysine food for each individual animal.

DISCUSSION.

The results of the experiments are shown in Charts I to VI. In all cases, the food intake was carefully measured, but in order to condense the data, it is not recorded for the individual animals in this paper. The rate of growth of the control rats on casein food was uniform and for the most part was practically the same as that of stock rats on milk, bread, and cabbage diets. Chart I shows the failure of any considerable growth on a gliadin diet. This is not due to a smaller food intake since the data indicated that there was an even greater food consumption per 100 gm. of rat on gliadin diets than on casein diets. That the failure of normal growth on gliadin diets continues for long periods is shown by Rats 37 and 48 (Chart I) which were studied for periods beyond those shown on the chart. These rats were kept on a lower level of protein intake and it is apparent that even with 15 per cent of gliadin, there is perfect maintenance and even a slight gain in weight. This question of protein level will be referred to later.

The curve of growth of Rat 15 (Chart I) shows very strikingly the effect of adding 0.5 per cent of lysine to the gliadin. This is not the result of an increased food intake, there being, in fact, a slightly decreased consumption of food. In this case, the lysine is equal to practically 2.8 per cent of the protein of the food mixture. The growth curves of other animals in subsequent

charts also illustrate clearly the growth-promoting effect of the addition of small amounts of lysine to gliadin diets. Attention should be called to the slow rate of growth of Rat 6 (Chart V, Diet K). The gliadin in this period was supplemented with impure lysine whose potency we wished to determine. This same sample of lysine was given to Rat 5 (Chart V, Diet K) with more satisfactory results. The results confirm the earlier work of Osborne and Mendel in which protein-free milk was used as the source of carbohydrate and inorganic salts and which has been questioned because of the possible occurrence of small amounts of nitrogenous compounds in the protein-free milk.² In the present series of experiments the only significant source of extra nitrogen is found in the nitrogen of the yeast vitamin powder which, in the amounts fed (50 mg. daily), would approximate 4 to 5 mg. of nitrogen daily.

The failure of α -hydroxycaproic acid to supplement gliadin as does lysine is indicated in Chart III. The rates of growth of rats on diets of gliadin supplemented with α -hydroxycaproic acid were not greatly different from those of rats on gliadin alone, whereas, when the same rats were given a gliadin plus 0.5 per cent lysine food, their gains in weight were quite pronounced. Rat 7 during 35 days on an 18 per cent gliadin diet made no gain in weight; during 42 days on a diet of 18 per cent gliadin plus 1 per cent α -hydroxycaproic acid there was a gain of only 7.5 gm.; on a 18 per cent gliadin diet plus 0.5 per cent lysine diet, there occurred in 32 days a gain of 58.5 gm. It may be argued that the rats ate less food while on the gliadin and gliadin plus α -hydroxycaproic acid diets than on gliadin plus lysine diets and that their failure to grow was due to a diminished food intake. However, in two cases, Rats 7 and 44, there was actually less food eaten per unit of body weight on a gliadin plus lysine diet than on gliadin plus α -hydroxycaproic acid diets, and in the

² Osborne and Mendel (Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1924, lix, 339) have recently discussed the supplementary value of "protein-free milk" and conclude that "obviously, therefore, no great part of the nitrogen of the protein-free milk belongs to any one of the essential amino acids; and, consequently, it cannot supplement to an important degree any amino acid deficiency of the proteins of the diet unless the latter are fed in very small proportion."

other rats of this series, while there was a slight increase in the amount of food consumed on the lysine diets, the increase in weight was entirely out of proportion to the increased food consumption. For example, Rat 13 (Chart III) during 42 days on a diet of 18 per cent gliadin plus 1 per cent α -hydroxycaproic acid showed a gain of 5.5 gm. with an average weekly food intake of 35.4 gm. In the next period of only 35 days, on a diet of 18 per cent gliadin and 0.5 per cent lysine, the food consumption was 5 gm. a week greater, but the gain in weight per 100 gm. of food was over six times that of the preceding period.

In view of the fact that gliadin contains a certain small percentage of lysine it was considered possible that rats fed the 18 per cent gliadin diets might not experience an acute shortage of lysine and that rats maintained on lower levels of gliadin where the deficiency in lysine was even greater than on an 18 per cent level, might develop a greater synthetic power of converting the caproic acid derivatives into lysine. For this reason, it was considered necessary to determine the minimum amount of gliadin, which, when supplemented with lysine, would allow rates of growth comparable to those on 18 per cent casein. Chart II summarizes the results. Rats 19 and 20 on 9 per cent gliadin diets show a comparatively rapid decline, especially in the case of Rat 19, although the food intake in both cases was satisfactory. When a 9 per cent gliadin food was supplemented with 1 per cent lysine, the rate of growth was greatly below that of a rat on 18 per cent gliadin plus 0.5 per cent lysine and only slightly greater than that of rats on 18 per cent gliadin alone. For example, Rat 21, on Diet B (Chart II), which consists of 9 per cent gliadin plus 1 per cent lysine, showed a gain of only 6 gm. in 35 days, only slightly greater than that of Rat 15 (Chart I) which made a gain in the first 35 days of 5.5 gm. on an 18 per cent gliadin diet. This same animal (Rat 15), on a diet of 18 per cent gliadin supplemented with 0.5 per cent lysine, gained 52.5 gm. in 35 days. It is evident that on a gliadin intake level of 9 per cent, other dietary inadequacies than lack of lysine become apparent, either an insufficient intake of total nitrogen or a deficiency in some other amino acid.

Rat 20, on a 12 per cent gliadin diet, made a slight increase in weight, 2 gm. in 42 days. However, when 12 per cent gliadin

was supplemented with 1 per cent lysine, the gain did not approximate that of control animals on a casein diet. This is further illustrated in the curves of growth for Rats 19, 22, 23, and 24. It was thought that failure of growth on these low gliadin levels might have also been due to a lack of cystine, or that an extra supply of cystine, because of its supposed relationship to oxidative function, might accelerate body metabolism and cause a more efficient utilization of gliadin. Rat 21 (Chart II, Diet C) shows that added cystine does not change the slope of the curve from that obtained with Diet B which contains 9 per cent gliadin with 1 per cent lysine. On a diet of 9 per cent gliadin plus 1 per cent cystine, there was practically no growth. It was found that a gliadin level of 15 per cent was adequate for maintenance and that this was the lowest level of gliadin which, when supplemented with 0.5 per cent lysine, would allow rapid growth. Subsequent to this phase in the work, all diets were prepared with 15 per cent gliadin.

There was available a limited amount of the sodium salt of ϵ -hydroxycaproic acid so that it was possible to conduct experiments on one rat (hooded rat) only. The result is shown on Chart IV, Rat 69. ϵ -hydroxy-, like α -hydroxycaproic acid, failed to influence the rate of growth.

When ϵ -aminocaproic acid was supplied with the gliadin, the gain in weight of rats was approximately the same as the gains on diets of gliadin alone (Chart V). When cystine was added to a diet of 18 per cent gliadin plus 1 per cent ϵ -aminocaproic acid (Rat 8, Diet O) it had no effect of causing a better utilization of this caproic acid derivative. However, substitution of lysine itself for the lysine derivative resulted in prompt growth. ϵ -Aminocaproic acid in these experiments, and α -aminocaproic acid, as previously shown (15), are evidently not available as precursors for lysine.

As with α -hydroxy-, ϵ -hydroxy-, and ϵ -aminocaproic acids, the results with α -hydroxy- ϵ -aminocaproic acid were negative (Chart VI). The addition of α -hydroxy- ϵ -aminocaproic acid failed to influence the rate of growth in any way, while, in the same rats, the response to the addition of lysine was satisfactory. The addition of cystine had no stimulatory effect, nor did it in any way effect the food intake (Rat 10, Chart VI).

The inability of the growing rat, under the conditions of these experiments, to synthesize lysine from the possible precursors studied, is not in accord with the perfusion experiments of Embden and Schmitz (12) nor does it lend support to the theory of protein synthesis from non-nitrogen rests and ammonium salts or urea as suggested by the feeding experiments of Abderhalden (14) and Grafe and Schl pfer (13). Theoretically, the results are disappointing. According to Neubauer (19) and others the first step in amino acid catabolism is conversion to the corresponding keto acid or keto aldehyde, and according to Kotake (20), under conditions of hydrolytic deamination, the corresponding hydroxy acid may also be formed. One would expect, under conditions in which there was a great demand for lysine as with the gliadin diets that, if the reversal of the hydrolytic deamination were at all possible, it should occur in this case. Equilibrium reactions, theoretically, are reversible, even in the living cell.

With α -hydroxy-, ϵ -hydroxy-, and ϵ -aminocaproic acids, a more complicated process of synthesis would be necessary to manufacture lysine and with these derivatives synthesis might not have been expected, especially in view of the fact that nor-leucine had been shown to be unavailable as a lysine substitute. However, with α -hydroxy- ϵ -aminocaproic acid, synthesis should be quite readily accomplished; the ϵ -amino group was already present and the amination of a hydroxy group has been shown to occur, at least in perfusion experiments. One may argue that the enzyme that caused a hydrolytic deamination could not reverse the process because of its intrinsic specificity, but there is existing evidence that certain lipases are able to reverse the process of saponification. Certain enzymes among the carbohydrate-splitting group are likewise capable of carrying out reversible reactions.

A possible explanation is that ammonia is not available in the same cell or in the same part of the cell in which construction of new tissue takes place. It is also possible that the conversion of ammonia into urea in the cells is so rapid that ammonia for amination of hydroxy groups is not available. This, however, hardly seems probable in view of the increased ammonia formation in response to acidosis. A non-absorption of the caproic acid derivatives might explain their failure to replace lysine in the

food. This is not likely in view of the close chemical relationship to lysine which is absorbed and utilized. Furthermore, Greenwald (21) proved that nor-leucine was absorbed and in normal animals yielded urea. In the phlorhizinized dog, its carbon was converted into glucose.

In the experiments described the α -hydroxy- ϵ -aminocaproic acid fed was optically inactive, the *dl* form. It was considered that the failure of lysine synthesis with this compound might have been due to the inability of the organism to utilize the *dl* form for synthesis. This explanation would be invalid if it could be shown that the organism of the rat could utilize *dl*-lysine as a supplement to gliadin instead of the naturally occurring *d*-lysine. The results of experiments with *dl*-lysine as a supplement to gliadin are shown in Charts III (Rat 17) and IV. The curves show a good utilization of the synthetic *dl*-lysine at levels of 1 per cent (equivalent to 0.5 per cent *d*-lysine). In the one experiment (Rat 68, Chart IV) in which 0.5 per cent of the *dl* compound was fed, growth did not occur as rapidly as with 0.5 per cent *d*-lysine. In the experiment with Rat 67 the food intake was greatly diminished during the period of the addition of the natural lysine to the diet, so that the failure of growth on this diet is readily explained.

The evidence presented seems to indicate that under the experimental conditions, lysine cannot be synthesized from closely related precursors. Furthermore, the evidence is not in support of the theory of a union between ammonia and the hydroxy groups of α -hydroxy fatty acids to form α -amino acids at least in the living organism. This is not in accord with the views of Embden and Schmitz (12) who perfused surviving organs, or of Abderhalden (14) and Grafe and Schläpfer (13) who studied the nitrogen balance after the administration of ammonium salts.

We believe, as the result of a careful consideration of the literature, that there is available no entirely satisfactory evidence of amino acid synthesis from ammonia and non-nitrogenous rests in the organism of the *living animal*.

SUMMARY.

1. The value of four caproic acid derivatives, α -hydroxycaproic, ϵ -hydroxycaproic, ϵ -aminocaproic, and α -hydroxy- ϵ -

aminocaproic acids, as supplements to the incomplete protein gliadin, has been studied in the white rat. None of these caproic acid derivatives could supply the deficiency of the protein (gliadin) element of the diet and promote growth as did lysine. They may not, therefore, be considered as available precursors of lysine.

2. Since growth was possible on diets of gliadin supplemented with *dl*-lysine, the failure of α -hydroxy- ϵ -aminocaproic acid to replace lysine for growth cannot be explained on the basis of the inability of the organism to utilize the *dl* form in body reactions.

3. The evidence presented indicates that under the experimental conditions of the present study, α -hydroxy acids are not capable of conversion by the animal organism into α -amino acids. This is not in accord with theories of conjugation of ammonia with a non-nitrogenous rest to form amino acids.

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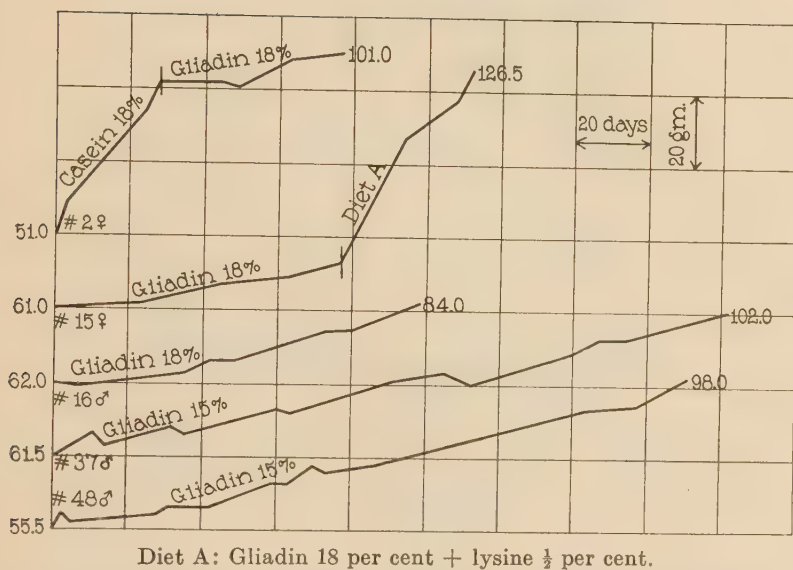
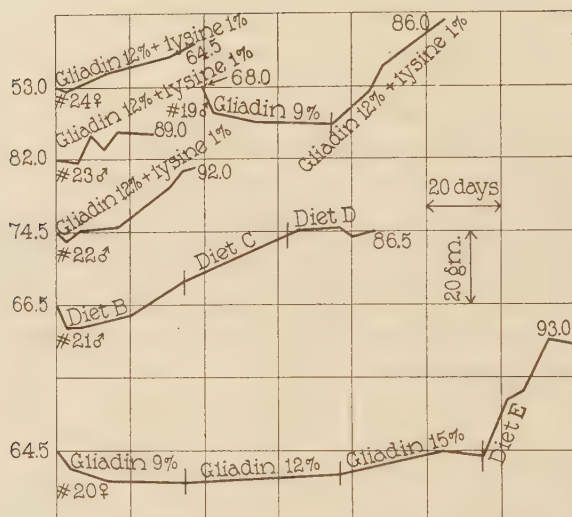


CHART I. Showing the curves of growth of rats on diets containing gliadin as the source of protein. It will be seen that there was some gain in weight, though the gains were not comparable to those with casein diets. The prompt response in growth when 0.5 per cent of natural lysine supplemented the gliadin is to be noted in the curve for Rat 15. With Rat 2, prompt cessation of growth in a fair sized rat was observed when casein was replaced by gliadin. The experiments with Rats 37 and 48 were continued beyond the period shown in the chart. At the end of 44 and 42 weeks, respectively, they weighed 111.0 gm. (Rat 37) and 118.0 gm. (Rat 48).



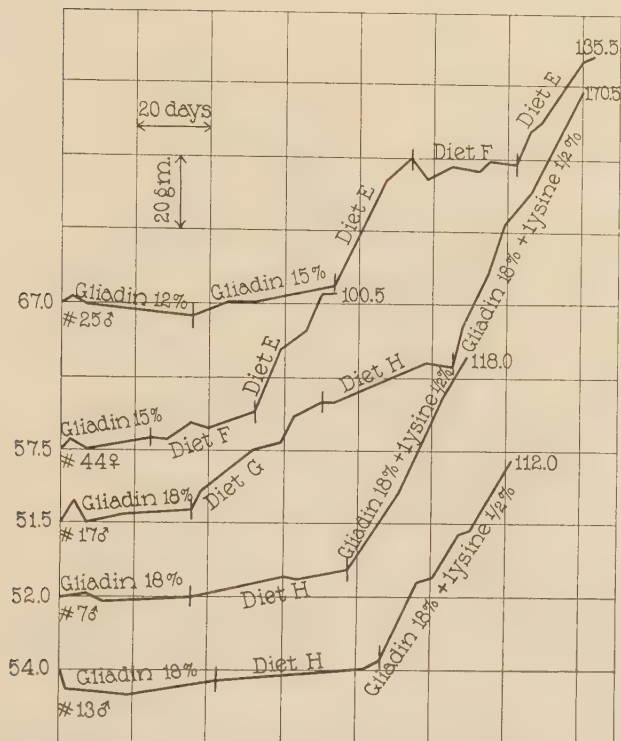
Diet B: Gliadin 9 per cent + lysine 1 per cent.

" C: " 9 " " + " 1 " " + cystine 1 per cent.

" D: " 9 " " + cystine 1 " "

" E: " 15 " " + lysine $\frac{1}{2}$ " "

CHART II. Showing the effect of low levels of intake of gliadin. Curves 19 and 20 show the inadequacy of gliadin for maintenance when fed at a level of 9 per cent of the total food. The replacement of this low protein intake by a higher level of 12 per cent gliadin (Curve 20) does insure maintenance, but inspection of Curves 22, 23, and 24 will show that when 12 per cent gliadin is supplemented with 1.0 per cent lysine normal growth fails to result. 9 per cent gliadin supplemented with 1.0 per cent lysine did not permit normal gain in weight (Curve 21, Diet B). The curve for Rat 21 (Diet C) shows that the addition of cystine to a 9 per cent gliadin + 1.0 per cent lysine food does not increase the rate of growth and demonstrates the prompt cessation of growth (Diet D) when the lysine was removed. Curve 20 shows that a 15 per cent intake of gliadin was quite sufficient for maintenance and when supplemented with lysine (Diet E) allows rapid growth.



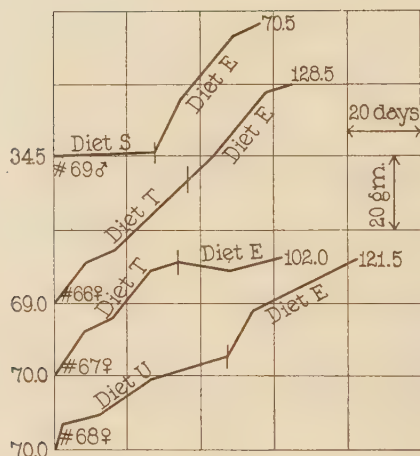
Diet E: Gliadin 15 per cent + lysine $\frac{1}{2}$ per cent.

" F: " 15 " " + α -hydroxycaproic acid 1 per cent.

" G: " 18 " " + synthetic *dl*-lysine 1 " "

" H: " 18 " " + α -hydroxycaproic acid 1 " "

CHART III. Showing the failure of α -hydroxycaproic acid to satisfactorily supplement gliadin on levels of 15 and 18 per cent protein intake. If either diet was supplemented with lysine, normal growth followed. Curve 17 (Diet G) shows the effect of supplementing 18 per cent gliadin with synthetic *dl*-lysine. Apparently, only the natural optical isomer is utilized completely. 1 gm. of synthetic *dl*-lysine dichloride was used, which is equivalent to 0.66 per cent of *dl*-lysine and only 0.33 per cent of the natural isomer, *d*-lysine. Compare also Chart IV.



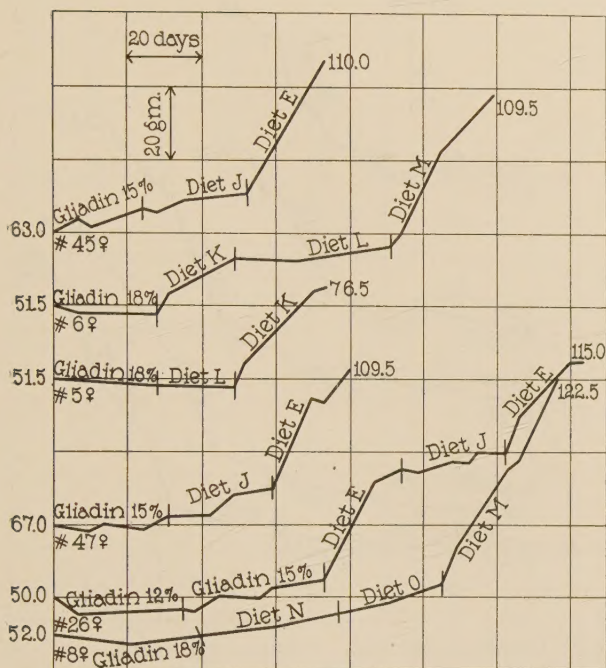
Diet E: Gliadin 15 per cent + lysine $\frac{1}{2}$ per cent.

" S: " 15 " " + ϵ -hydroxycaproic acid 1 per cent.

" T: " 15 " " + synthetic *dl*-lysine 1 " "

" U: " 15 " " + " " $\frac{1}{2}$ " "

CHART IV. Showing the failure of ϵ -hydroxycaproic acid (Diet S) to supplement gliadin and the response when it was replaced by lysine (Diet E). Curves 66 and 67 show the growth of rats on diets of 15 per cent gliadin plus 1 per cent *dl*-lysine (Diet T) and the response when this diet is replaced by 15 per cent gliadin plus 0.5 per cent *d*-lysine (Diet E). The failure of growth of Rat 67 on 0.5 per cent *d*-lysine is ascribed to a diminished food intake. Curve 68 shows the growth on 0.5 per cent *dl*-lysine (Diet U) and the subsequent growth on 0.5 per cent *d*-lysine. Growth on the latter diet is somewhat greater. Compare also Rat 17, Chart III.



Diet E: Gliadin 15 per cent + lysine $\frac{1}{2}$ per cent.

" J: " 15 " " + ϵ -aminocaproic acid 1 per cent,

" K: " 18 " " + lysine (?) 1 per cent.

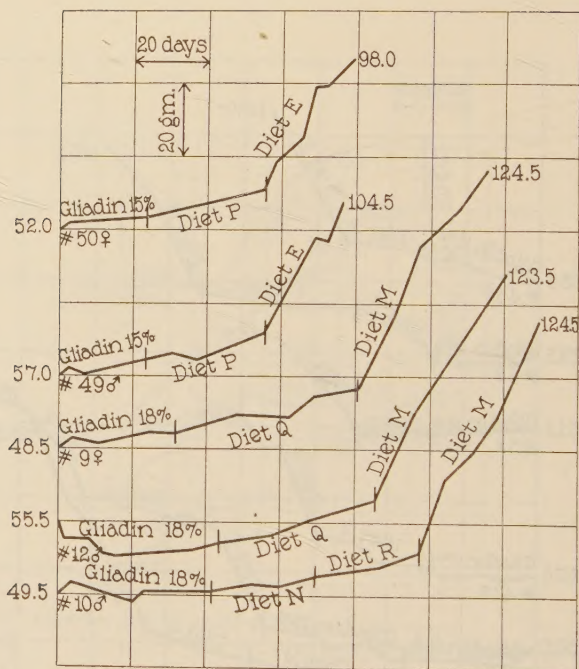
" L: " 18 " " + ϵ -aminocaproic acid 1 per cent.

" M: " 18 " " + lysine $\frac{1}{2}$ per cent.

" N: " 18 " " + cystine 1 " "

" O: " 18 " " + " 1 " " + ϵ -aminocaproic acid 1 per cent.

CHART V. Showing that the rate of growth is not altered when ϵ -aminocaproic acid supplemented the gliadin of the diet at levels of 15 and 18 per cent of protein intake, and the failure to utilize this acid in place of lysine.



Diet E: Gliadin 15 per cent + lysine $\frac{1}{2}$ per cent.

" M: " 18 " " + " $\frac{1}{2}$ " "

" N: " 18 " " + cystine 1 " "

" P: " 15 " " + α -hydroxy- ϵ -aminocaproic acid 1 per cent.

Diet Q: Gliadin 18 per cent + α -hydroxy- ϵ -aminocaproic acid 1 per cent.

Diet R: Gliadin 18 per cent + α -hydroxy- ϵ -aminocaproic acid 1 per cent + cystine 1 per cent.

CHART VI. Showing the effect of adding α -hydroxy- ϵ -aminocaproic acid to gliadin diets. This derivative, even though very closely related to lysine, fails to influence the rate of growth as does lysine, thus demonstrating failure to aminize the α -hydroxy groups even under stress. Substitution of lysine (Diet M) for the other caproic acid derivative increased the rate of growth in all cases. Addition of cystine (Diet R) did not facilitate lysine synthesis from α -hydroxy- ϵ -aminocaproic acid.

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BALTIMORE, U. S. A.